DROSOPHILA AMPELOPHILA LOEW BRED IN THE DARK FOR SIXTY-NINE GENERATIONS.[1]

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In a short note (BIOL. BULL., '10) I stated that I had been breeding *Drosophila* in the dark for forty-nine generations or more than two years; that the darkness had produced no visible effect either in the color of the body or in the structure of the eye; but that there seemed to be a difference in their reactions to light, the ones bred in the dark reacting more slowly than those bred in the light. The evidence on which this last statement was based was meager. It was obtained by placing a number of flies in a clean vial and then revolving it so as to bring first one end and then the other toward the light. In this way it was demonstrated that the flies bred in the dark were noticeably much slower in their reactions than those bred in the light. These observations as far as they went were correct, but as we shall see they were not extensive enough and hence led to an erroneous conception.

I have just finished an experiment in which each fly was tested individually. This experiment was based upon four series of flies, 1,000 in each series: first (series no. 1), those bred in the dark for sixty-nine generations; second (series no. 2), those bred in the dark for sixty-four generations and then placed in the light for six generations; third (series no. 3), those bred only in the light; and fourth (series no. 4), a new strain from series no. 3 bred in the dark for five generations.

The apparatus for testing each fly consisted of a Welsbach lamp, a heat screen three inches thick and a glass tube one inch inside diameter and nine and three eighths inches long. The apparatus was arranged as shown in the diagram (Fig. 1), the end of the tube toward the light being ten and three fourths inches from it. The glass tube was perfectly clean on the inside and wrapped with black cloth on the outside. The flies were

[1] Contribution from the Zoölogical Laboratory of Indiana University, No. 120.

introduced into this tube by means of a shell vial three and one half inches in length and large enough to slide over the end of the glass tube. The end and about one half inch of the side of this vial were also covered with black cloth so when the vial was slipped over the tube, the tube admitted no light except at the end toward the lamp. By means of a stop watch I obtained the time it took each fly to travel the distance of nine and three eighths inches toward the light. Sometimes the flies did not react and in such cases they were counted out at the end of one minute. Records of these flies were also kept. This limit was placed at one minute, as experience showed that if they did not react within this time they might not react for five or ten

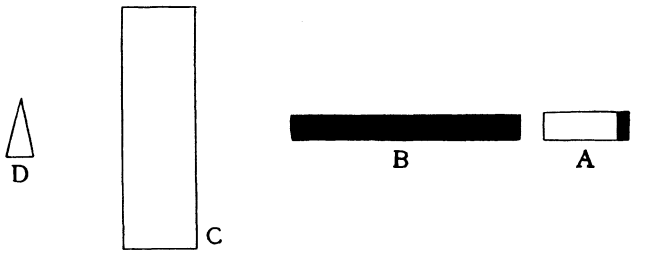

FIG. 1. Arrangement of the apparatus. *A*, the shell vial, three and one half nches in length; *B*, the glass tube, nine and three eighths inches long; *C*, the heat screen, three inches thick; *D*, the light, ten and three fourths inches from the end of the glass tube. The parts colored black were wrapped with black cloth.

minutes, or even longer. Extreme care was used to have external conditions as nearly uniform as possible. The temperature of the room at the time of testing varied between 74 and 78 degrees Fahr., but I could not see that a variation within this range made any difference. All flies were changed to perfectly clean vials and allowed to stand from ten to thirty minutes before testing, so they might become accustomed to a clean surface such as the inner surface of the tube into which they were introduced. The flies bred in the dark were placed in the light during this ten to thirty minutes in order to overcome any temporary effect of the darkness. All flies were tested at approximately the same age, from six to twenty-four hours after hatching. They were fed on banana which was permitted to reach about the same degree of fermentation before using.

With these precautions it was found that offspring of different parents within the same strain, those bred in the light as well as in the dark, vary considerably in their reactions. Further, this difference does not seem to be due to inheritance from the parents. To test this I selected five flies, each of which failed to pass through the tube in five minutes. The next day, I retested these same five flies and again they failed to react. This sluggishness was not due to poor development, as they looked strong and vigorous. These five flies were bred and their offspring tested. The average time of those which passed through the tube and the percentage which failed was approximately the same as for the series, indicating that something other than inheritance is the cause of this difference in reaction. I have taken two pairs, brothers and sisters, fed them on the same food, kept them side by side in the same room and yet their offspring showed a decided difference in their reactions to light. This fact has caused me a great deal of trouble and led to the erroneous conception stated in the previous note. In fact I can obtain the same result between the offspring of different parents in the same strain which I formerly stated was obtained by comparing flies bred in the dark with those bred in the light. Often this difference is so marked that it is noticeable before the flies are tested, those reacting least being more easily excited by handling. The only possible cause of this difference which I have to suggest is the food, as it may undergo different degrees of fermentation after it is placed in the vial. Another thing which leads me to believe that the food may be the underlying cause is that the last flies taken from any one vial are usually more sluggish than the first ones. Even with this difference in the reactions of the offspring of different parents, I believe the reactions of 1,000 individuals from many different parents gives a close approximation to the average for the strain.

The following table gives the average time of the flies in passing

1,000 Flies in Each Series.	Average Time of Flies which Passed through the Tube.	Percentage of Flies which Failed to Pass through the Tube in One Minute.
Series No. 1.....	13.9 seconds.	25.1 per cent.
Series No. 2.....	14.24 seconds.	23.6 per cent.
Series No. 3.....	17.62 seconds.	29.5 per cent.
Series No. 4.....	15.89 seconds.	23.2 per cent.

through the tube and the percentage which failed to pass through in one minute.

This table shows that the average time of the flies bred in the dark for sixty-nine generations (series no. 1) is least, 13.9 seconds, and the average of those bred only in the light (series no. 3) is greatest, 17.62 seconds. This is a difference of 2.72 seconds in favor of the flies bred in the dark. Likewise the percentage of flies which failed to pass through the tube in one minute is less (25.1 per cent.) in series no. 1 than in series no. 3 (29.5 per cent.). Series nos. 2 and 4 were run as controls and the average time is intermediate between the two extremes, while the percentage which failed to go through the tube is less than in series nos. 1 and 3. However, I do not believe the difference between any of the series great enough to be of any special significance and the only conclusion which can be drawn is that the darkness operating through a period of sixty-nine generations has produced no visible effect in the reactions of *Drosophila* to the light of a Welsbach burner. It should be pointed out, however, that the only cave condition present in the experiment is darkness; that it is possible and indeed probable that factors other than darkness (constant temperature and moisture) may play some part in the changes which have taken place in cave animals. If this be true the real test comes not in rearing animals in the darkness but in a true cave environment.

That the constancy of the environment may be a potent factor in the production of degeneration in cave animals is strengthened by the recent unpublished experiments of Tower. In a letter, August 2, 1911, Professor Tower makes the following statement:[1] "It has been my experience that there is hardly anything so injurious to breeding stock as a constant environment. It will produce degeneration, reduced activity, and not infrequently will result in actual elimination of the race." Also in the experiments of Calkins and others on protozoa, it seems that the constancy of the food supply and the constancy of the chemical make up of the medium in which they lived, brought about, at least a shorter cycle than occurs in a food supply and

[1] I wish to thank Professor Tower for the permission to use this statement before it has appeared in print.

medium which are changed at intervals as shown by Woodruff's experiment (*Archiv für Protistenkunde*, '11).

In caves conditions are practically uniform. Also in dark corners under stones and logs, conditions are more nearly uniform than in the open. Since it seems almost certain that forms which now inhabit caves once lived outside under stones and in dark corners and that they had varied in the direction of cave animals before they entered caves, is it not possible that the more nearly constant environment of the dark corners under stones and logs has started the degenerative changes which have been carried to their present condition in the more constant environment of the cave. To be sure all changes in cave animals are not degenerative. The tactile sense, for example, becomes highly developed, but no doubt a second causative factor enters here.